Corneal structure, transparency, thickness and optical density (densitometry), especially as relevant to contact lens wear – a review

Doughty, Michael J.; Jonuscheit, Sven

Published in:
Contact Lens and Anterior Eye

DOI:
10.1016/j.clae.2018.11.014

Publication date:
2019

Document Version
Author accepted manuscript

Corneal structure, transparency, thickness and optical density (densitometry), especially as relevant to contact lens wear – a review

Michael J Doughty, Sven Jonuscheit

Vision Sciences, Department of Life Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 OBA

Corresponding author: sven.jonuscheit@gcu.ac.uk

ABSTRACT 188

Clinical instruments using Scheimpflug image-based methods to obtain optical sectional images of the cornea have been introduced in recent years along with proposals that it should be possible to routinely and reliably measure the optical density (referred to as the densitometry) of the human cornea in situ. Such a concept is reviewed from the perspective of what might be considered as the basic principles underlying the understanding of corneal transparency (from the 1950's) and the progressive changes in these ideas from subjective slitlamp-based clinical observations from the late 1960's, especially in contact lens wearers. Much more has been learned about the overall macrostructure (including corneal thickness) and the ultrastructure of the cornea from contemporary studies in the 1990's, and these aspects of the cornea will be reviewed alongside consideration of the methods of assessing the optical characteristics of the cornea in the living eye. From these perspectives, in this review systematic consideration will be given to what objective (quantitative) output one of these Scheimpflug-based systems provides and how this information might be actually related

to corneal transparency characteristics that might be observed clinically, particularly after long-term contact lens wear.

Keywords: cornea, human, contact lens wear, corneal oedema, cornea swelling, corneal opacities, corneal densitometry

1. **Introduction**

The normal (healthy) mammalian cornea is optically transparent (i.e. transmits a large proportion of visible light) as a consequence of its unique structural organization. This optical clarity can be compromised in contact lens wear. Subjective slitlamp-based assessments of this have been undertaken for many years, perhaps along with more specific comments on the localization of any alterations in transparency (seen as haze or discrete opacities), and later augmented by measurements of corneal thickness by pachymetry and functional assessments of vision such as contrast sensitivity. A newer concept has been developed by which an objective optical Scheimpflug imaging approach can be used to assess any differences in transparency or optical density, presented in terms of optical densitometry units. In the present review, transparency of the cornea will be considered from a basic laboratory perspective as well as from clinical assessments of its appearance and functional transparency. From this perspective, a newer proposal for application of an *in vivo* optical density assessment will be considered, especially within the context of assessing the impact of contact lens wear on the human cornea.

2. Cornea structure and transparency

The mammalian cornea is composed of several distinct layers (epithelium, Bowman's layer, the stroma containing keratocyte cells, Desçemet's layer and the endothelium) with their overall transparency (or clarity) generally being considered to be an essential aspect of high resolution vision [1,2], providing there is also a stable pre-corneal tear film [3]. In a normal healthy cornea, the anterior cell layers of the stratified squamous epithelium are transparent and essentially invisible to transmitted broad-wavelength (white) light and so should appear largely featureless and optically transparent when viewed in optical section in a slitlamp [4,5].

In early perspectives on corneal transparency, the interest was mainly on the role of the corneal stroma as the primary determinant of optical transparency [6]. The bulk of the human cornea is made up by the stroma which is composed of some 200 flattened sheets (lamellae) of collagen fibrils [1,7]. A hypothesis was developed in which the optical transparency (of the stroma) would be predictably dependent upon the degree (extent) of order of the fibrils within the lamellae [6]. Modelling studies, undertaken in more recent years to support such ideas, have been presented to indicate that light scattering as associated with variations in fibril spacing could be predictably higher in the anterior stroma [85]. From a clinical perspective, especially that which was likely dominant in the 1950's and 1960's, disorganization of collagen fibrils occurred when there was development of substantial oedema (swelling) of the cornea [6,9]. Using slitlamp-based assessments, the overall oedema and particularly in its posterior location (within the cornea) was likely evident and so such a scenario for the development of corneal oedema and scattering of light by the cornea was generally considered to be a likely consequence of any dysfunction or even some type of damage to the posterior corneal endothelium. However, even some early laboratory studies on excised corneas [10] and early clinical slitlamp-based observations of oedematous corneas in the 1960's and 1970's [4,11] indicated that another important source of white light scatter is within the corneal epithelium. In the 1990's, evidence was provided that keratocytes within the corneal stroma also could play an important role in determining what the overall light scatter and thus the transparency of the cornea might be [12].

In macroscopic terms, a normal cornea appears to be optically transparent, as evidenced by the fact that substantial detail can be seen of the iris tissue in front of the crystalline lens in both humans and animals when examined from outside. Actual

measurements of freshly excised corneas can be used both to illustrate this transparency, as well as to demonstrate just how readily it can change [13] (Figure 1).

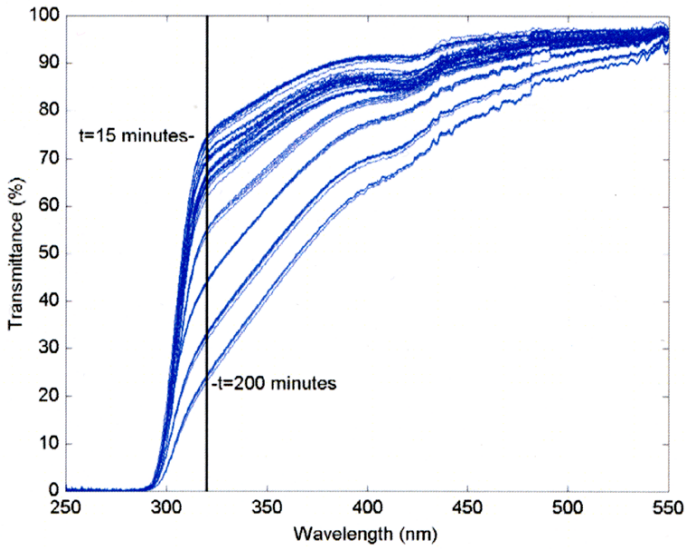

FIGURE 1 Representative UV-visible light transmission profiles taken through freshly isolated rabbit cornea at room temperature (top curve, 15 minutes) and the resultant rapid changes over a period of 200 minutes after oedema (swelling) was allowed to develop *ex vivo*. From ref. 13; copyright IOP Publishing.

For example, at wavelengths above 400 nm, a fresh rabbit cornea (processed as quickly as possible post-mortem, in this example within 15 minutes) can be shown to transmit just over 90 % of incident light at 400 nm and only slightly less than this (down to 75 %) in the UV-A region of the spectrum [13]. However, with, for example, short periods of keeping an excised rabbit cornea on ice can slightly reduce the net light transmission profile by a few percent [14], and considerably lower net transmission at 400 nm (i.e. just over 50 %) has been reported for slaughterhouse-procured rabbit corneas where there was a quite considerable delay between procurement and spectral measurements being taken [15]. These differences

are a predictable consequence of slight to rather substantial post-mortem changes. These types of reductions in optical transparency of an excised cornea are also illustrated in Figure 1 as the sequence of scans over time at room temperature. With such an excised cornea being repeatedly wetted to stop any desiccation *ex vivo*, it absorbs fluid and swells rapidly with very obvious and substantial decreases in light transmission at lower wavelengths. After 200 minutes, this light scattering (with reduced transmission) can be especially evident in the 320 to 400 nm waveband and is noticeably visible as a (bluish) haze to the excised cornea.

In somewhat older studies, now using slaughterhouse-procured bovine (cow) corneas, measurements of white light transmission were made with a photometer to also illustrate how substantial the swelling-related changes in a cornea could be [10]. Attempts were made to relate the changes in 'specular density' of the excised corneas to alterations in corneal thickness [10]. The actual results from the experiments were essentially inconclusive, and likely due to substantial difficulties with both photometer calibration and obtaining reliable thickness measurements with callipers. Notwithstanding, while actual measurements of backscattered versus forward scattered light were not possible, such early studies gave quite considerable thought to why the transmitted light should change. However, in later *in vivo* studies, measurements were taken that indicated that the overall light scattering properties of the cornea could be shown to be dependent on the extent of swelling (oedema), in these cases as associated with cataract surgery [16].

While laboratory studies can be used to demonstrate just how readily corneal transparency can be reduced, the type of spectral analyses shown in Figure 1 do not indicate why the optical clarity is reduced. As indicated earlier, the once dominant theory was that the changes were the result of disruption of the uniform collagen fibril spacing, but more contemporary ideas also consider the keratocytes as an important source of light scatter. As

assessed at the resolution available in a spectrophotometer or some type of photometer or indeed clinical slitlamp-based assessments of corneal optical sections, very little detail of the corneal stroma is evident. That the stroma would be simply considered as a homogeneous or slightly heterogeneous structure that changed as swelling (oedema) developed is very much in line with earlier concepts on corneal transparency that essentially ignored the keratocytes. However, higher resolution options are available in the form of confocal microscopy (CFM; Figure 2) [2]. With careful adjustment of the instrument, this method of looking through the corneal stroma can be used in a 'through-focussing' mode to reveal the subtle features of the keratocyte cell bodies at different depths (Figure 2), as well as facilitate actual thickness (depth) measurements [17]. In such a coronal view (i.e. looking through the corneal layers and the keratocytes), it is mainly the nuclei of the cells that are evident which do scatter a small amount of light. In addition, discrete micro-opacities with sizes much smaller than the nuclei have also been observed by CFM in normal human corneas [18-20]. Using the collected data from different numbers of layers assessed by CFM, estimates can be made of the total amount of light scatter even in normal corneas [21]. Under conditions where there is considered to be substantial stress on these cells (e.g. after photorefractive keratectomy), the keratocytes can change their shape and their cytoplasm now scatter much more light to the extent that (anterior) cornea haze would be evident [22].

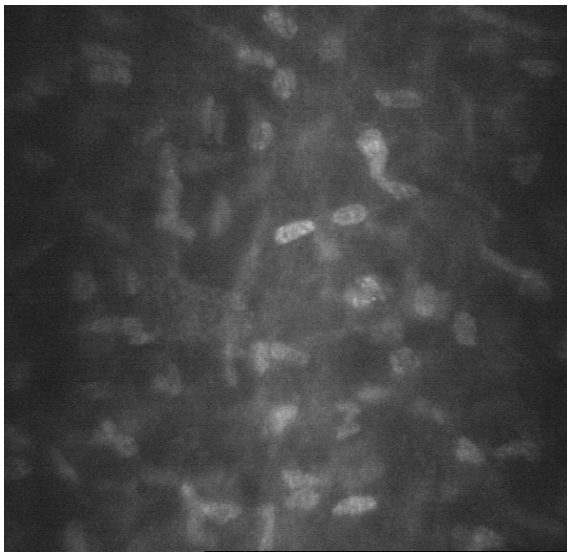

FIGURE 2 In vivo confocal microscopy image a human cornea to illustrate the light scattering (as brighter regions) associated with the nuclei of the keratocytes within the corneal stroma. From ref. 2; copyright Elsevier.

Even more cellular detail can be seen using transmission electron microscopy (TEM) on excised corneas that have been specially processed with electron dense stains. Following embedding in special resins, very thin sections can be made though the stroma from either the same perspective as that viewed in confocal microscopy (i.e. a coronal view) or from what can be considered as equivalent to an optical section side view (i.e. sagittal view) with the same being used in conventional histology evaluations of the cornea. For the TEM, the nucleoproteins and nucleic acids of nuclei preferentially absorb (bind) more of the heavy metals used to stain the tissue sections, as compared to less absorption of the surrounding collagen fibrils.

The coronal sections can be used to look through the keratocyte cell nuclei (Figure 3). In freshly-prepared normal tissue, the smooth edges and roundish to slightly-oblate shape to the nuclei can be seen because of the relatively high density of the staining, as compared to

the surrounding cell cytoplasm (not usually evident in the confocal microscopy), a density that is presumably sufficient to impair light transmission and so scatter light in a somewhat unpredictable way even though the nucleus only appears to occupy approximately one third of the cell body [23]. Surrounding the edges of the cell, but not resolved at the magnification used in the image, are the collagen fibrils extending from the keratocytes (to give a lighter grey background).

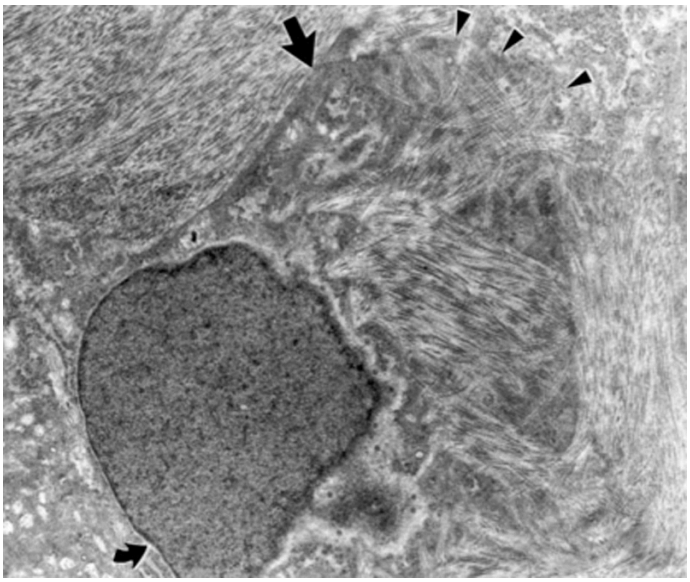

Figure 3. Transmission electron microscopy (TEM) image of a coronal section through the nucleus and cell body of a rabbit keratocyte. Part of the cell body edge is identified by the straight arrow to distinguish it from the often more prominent edge of the cell nucleus indicated by the curved arrow. The arrowhead show where parts of the cell membrane are obscured by the many collagen fibrils emerging from the cell. From ref. 23; copyright Elsevier.

If conventional sagittal sections are made for TEM assessments of the keratocytes, then the nuclei can be expected to be a very dominant feature [1,24]. As shown in Figure 4A,

the keratocyte in 'normal' cornea can be expected to stains relatively densely principally because only a small profile of the flattened cell is seen and it is dominated by the nucleus. The surrounding cell cytoplasm around the nucleus is often very thin (attenuated) when viewed from this perspective and often only small parts of this may be seen, perhaps with some small vacuoles in the cytoplasm being evident. Overall, the cell body of the keratocytes can extends to quite substantial distances away from the nucleus.

Such sagittal sections can also be used to illustrate changes in the density of the keratocyte nuclei (Figure 4B). In this example, the cell is identifiable only from the many vesicular remnants surrounding collapsed nucleus which is considerably shrunken with the condensation of the nucleoproteins and nucleic acids resulting in even more heavy metals being absorbed during the staining when the cells show apoptotic (necrotic) changes. If this chromatin condensation was present in the cornea *in situ*, then it might be expected to be denser and scatter more light.

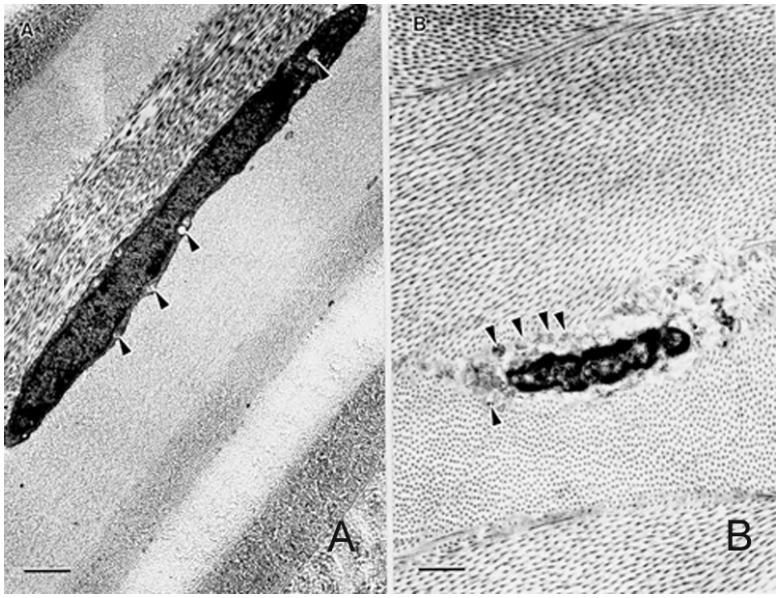

Figure 4. Transmission electron microscopy (TEM) image of sagittal sections through a normal-appearing keratocyte (A) or one shrunken after presumed apoptosis (B). The arrowheads in (A)

indicate small vesicles which can be present despite an intact cell membrane, while those in (B) indicate a cell composed of many vesicular remnants along with the dense remains of condensed chromatin of the nucleus. The separation of the collagen fibrils when oedema develops is evident in (B). From ref. 24; copyright Taylor & Francis.

With the sagittal view of the corneal stroma that can be obtained with TEM, even at relatively low magnifications (Figure 4A), the layers (lamellae) of the corneal stroma can be seen as bands with slightly different overall staining. This heterogeneity results from whether or not the collagen fibrils in individual lamellae are cut through perpendicularly (and seen in 'cross section' with a round profile) or longitudinally (and seen as long fibrils with a certain width), with some lamellae being (unavoidably) cut through at intermediate angles. The resolution of the fibrils, cut through in either perspective, can be especially evident when oedema develops (as shown in Figure 4B) with individual collagen fibrils being clearly evident. While pachymetry measures were not made on this specimen, it is likely that at least a 50 % increase in corneal thickness had occurred. As outlined earlier, it is generally considered that the disorganization of the fibrils within individual lamellae results in increased light scatter.

3. The normal human cornea and its assessment *in vivo*

Overall, the cellular (or fibril) detail that can be seen with CFM or TEM is not evident in most *in vivo* assessments of the cornea. Notwithstanding, an optical section of the cornea viewed with modern day photo-slitlamps can be readily captured as a (fairly) high resolution image suitable for some type of analysis of transparency, clarity, opacity or density [25-27]; such assessments required customized image acquisition and special image-processing software. Similar approaches have been applied to analysis of corneal images obtained by confocal

microscopy, including those from contact lens wearers [28]. This principle was applied in early studies as well, with it being noted that correct alignment of repeat images was a challenging aspect of such assessments as well as how to assign a particular grade to any opacities observed or recorded [16]. In recent years, however, a user-friendly and largely automated image capture and processing system has become available as a clinical instrument commonly referred to by its trade name, Pentacam. An output measure, of what is referred to as the corneal (optical) density, has been presented as the modern day approach to assessing corneal transparency and thus the overall 'health' of the cornea [29,30]. . As noted earlier, a normal cornea should be essentially optically transparent. When viewed in optical section by some type of slitlamp-based biomicroscopy then – essentially – nothing substantial should be visible [4,5].

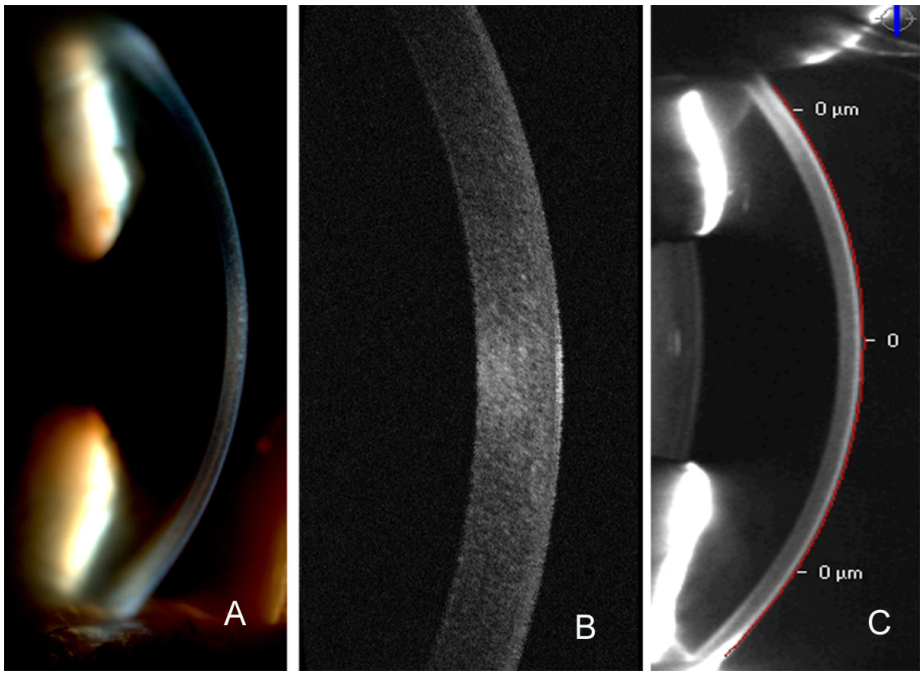

Figure 5. Optical sections taken through the cornea of the same adult individual in vivo by three different instruments (A) conventional photo-slitlamp, (B) optical coherence tomography (OCT) and (C) by the Pentacam Scheimpflug photography system.

Notwithstanding careful slitlamp examination of what might be considered to be a reasonably healthy cornea can reveal some subtle and discrete opacities (Figure 5A). The location of these can usually be ascertained by appropriate focussing (at least on the living eye) and in the photograph shown these appear to be more in anterior corneal stroma. Such 'opacities' or discrete areas reflecting the white light could also be in the tear film and asking a patient to blink a couple of times may be useful to assess if this is the case. As shown in Figure 5A, the slitlamp optical section should highlight (or define) the anterior and posterior surfaces of the cornea, sometimes with the anterior tear film-epithelial interface perhaps being more prone to significant artefacts.

The slitlamp is however just one way in which a cornea can be viewed in optical section. As viewed with specially collimated white light as used in some types of optical coherence tomography (OCT) instruments then the anterior surface may be apparently resolved as a layer (or region) showing focal light scatter not eminently visible in the slitlamp (Figure 5B). The image shown was taken from the same cornea illustrated in Figure 5A. The OCT image appears to show a very thin reflective layer at the anterior surface which, because of the known thickness of the cornea shown (of 0.510 mm), is unlikely to be the corneal epithelium or even the most anterior layers of the epithelium but rather the pre-corneal tear film. However, with the use of some types of laser illumination for OCT, this anterior layer visualization can be very noticeable and considered to be the corneal epithelium itself (as well as the tear film) [31]. The OCT image shown in Figure 5B also appears to show a modest number of discrete opacities that appear to be well within the depth of the central stroma, with these perhaps being accentuated by the higher illumination of this region of the stroma.

As with slitlamp observations, the anterior and posterior surfaces of the cornea can be expected to be well defined in OCT imaging.

For the Pentacam system, an optical section through the cornea also defines the anterior and posterior surfaces, with the instrument software applying a metric (shown as a slightly irregular red line in a colour image) to show the location of the anterior interface (Figure 5C). From such locations, the instrument can be simply used to provide thickness values across the cornea [32]. It should be noted however that the light scatter from the anterior aspect of the cornea (which is the same viewed in the other figures) is more substantial, extending beyond where the actual corneal epithelium would be expected to be, i.e. is an anterior portion of the cornea comprising both epithelium and anterior stroma. The reason why this image results from Scheimflug imaging of the cornea does not appear to have been explained in the peer-reviewed literature. Notwithstanding, a comparison can be made to the posterior aspect of the cornea which, in this example, shows less light scattering.

4. The human cornea response to contact lens wear

As indicated earlier, clinical observations made in the 1960's and 1970's very much served to highlight problems associated with early contact lens wear; induced swelling (oedema) of the cornea was evident [4,33]. Patients can report visual blur along with (coloured) haloes when looking towards distant light sources [34]. At least over the short term (i.e. a few hours), slitlamp-based observations indicate that oedema of the cornea is likely to start at the location most substantially affected by the presence of a contact lens (i.e. the corneal epithelium) with this being the likely cause of visual decrement (including haloes around lights, etc) [35-37]. Such epithelial oedema may have actually been apparent in many early studies although it is hard to find specific comments or notes on this, perhaps because

attention was given to overall pachymetry as the more useful indicator or the presence of oedema. It was recognised that the overall development of corneal oedema could be assessed by measures of corneal thickness and so started the routine use of pachymetry to assess the impact of contact lens wear on the cornea.

It is now well established that corneal thickness is a useful indicator of ocular health as decreased clarity is expected with increased corneal thickness [38,39]. For corneal thickness, particularly central corneal thickness (CCT), various studies indicate that it is either constant in adult Caucasian individuals or can decline with respect to age especially in Asians [39-43]. Overall, studies indicate that a small amount of oedema could be tolerated if contact lens wear was to be sustained on a day-to-day basis with only slight increases in CCT generally being observed in adapted contact lens wearers [11,39,44]. In contrast, CCT has been reported to be reduced in some subjects wearing soft or RGP contact lenses with there being a trend for decreasing thickness with years of lens wear [45]. However, no obvious difference in CCT were noted in newer studies assessing long term (average 16 y) soft contact lens wearers compared to non-lens wearers [46].

From a perspective of the visual consequences of corneal oedema, assessments of contrast sensitivity function have been considered to be useful. To see clearly, there should be no significant light scatter from the cornea or the crystalline lens inside the eye (cataracts). If there is some light scatter, however, then the ability to distinguish fine (line) details is expected to diminish [36,47-49]. This is expected to occur with age, especially after 40 years, and can be assessed using contrast sensitivity function (CSF). Studies on contact lens wearers have however yielded inconsistent results in terms of the magnitude of any decrement and the wavelength at which it was most evident [50-53].

For longer term contact lens wear, other types of changes in the cornea are possible. At least for older hydrogel contact lens wear, slitlamp-based assessments indicated the development of discrete (micro) opacities principally in the corneal stroma, although it may rather difficult to judge the location (depth) of such changes and / or grade them in routine slitlamp-based assessments [54-58]. Such micro-opacities may be more noticeable when some corneal swelling is evident since some of these changes were associated with modest (but chronic) oedema to the extent that striae could be evident thus indicating that the more posterior aspect of the corneal stroma was affected. With a much higher resolution assessments by confocal microscopy (CFM), even smaller opacities have been observed in modern-day contact lens wearers [59-62], and referred to as 'microdots' or 'white dots'. These may be more obvious in the posterior stroma and so be linked to posterior stromal oedema [62]. Similarly, general increases in the scattered light was reported to be observed at all depths of the cornea in contact lens wearers compared to non-lens wearing individuals [28].

The other major change in the cornea associated with contact lens wear is the development of changes in the corneal endothelium with the alteration in the uniformity of the cell mosaic now generally referred to as polymegethism (and reported as the coefficient of variation or CV in the cells areas). While the changes can be very notable, it has yet to be established whether the presence of (substantial) polymegethism has any predictable clinical consequence for the contact lens wearer. Stated another way, the extent of polymegethism does not appear to have a predictable effect on corneal thickness, for example [63].

5. Towards objective assessment of corneal clarity by densitometry and relevance to contact lens wear

If the cornea is viewed with narrow-waveband coherent (laser) light such as used in Optical Coherence Tomography (OCT) then the more anterior cell layers of the cornea can be notably visible in optical section [30]. If the cells develop oedema then this swelling can produce a hazy appearance to the corneal epithelium as a result of backscattered light [64-66].

One approach to measurement of backscattered light from the cornea *in vivo* is to use Scheimpflug imaging. In early applications of the technique, it was noted that more backscattered light appeared to be reflected of the anterior and posterior surfaces of the cornea rather than from the corneal stroma [67]. However, with development of newer illumination techniques, this is not generally seen but rather that the backscattered light is more pronounced from the anterior portion of the cornea (see Figures 5 and 6).

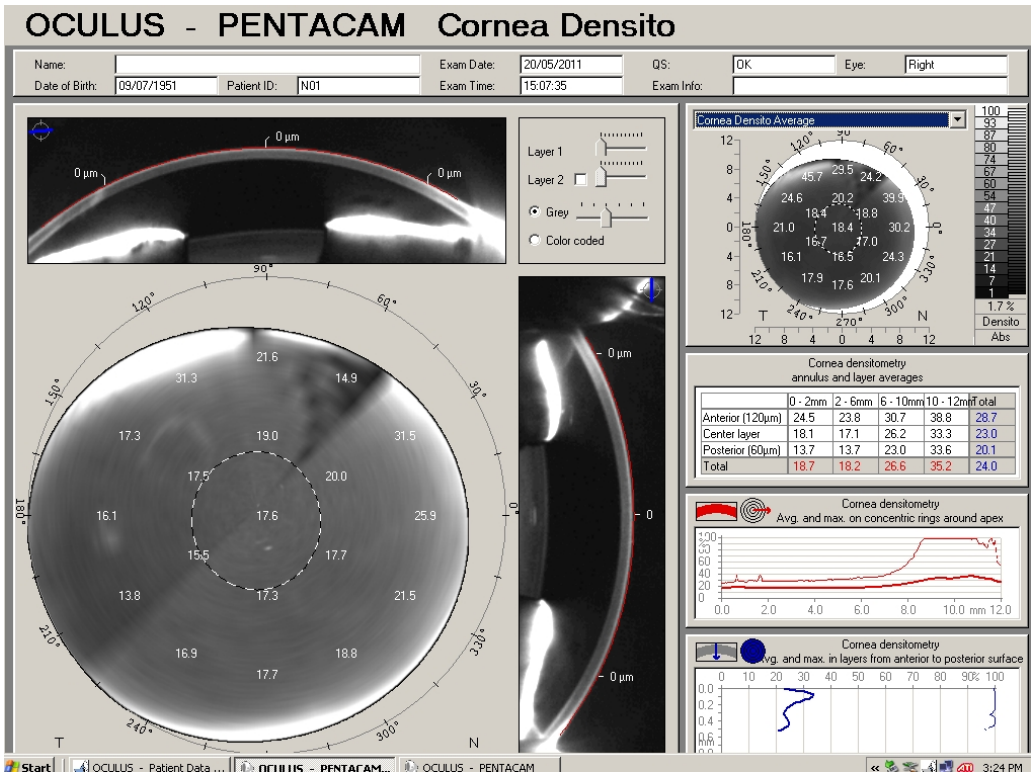

Figure 6. Complete densitometry data output screen from the Pentacam Scheimpflug photography system showing the profiles of the cornea and the associated estimates of density as a topical graphical map (top right; the Cornea Densito Average) and as a set of numbers in gray scale units for different locations (middle right, Corneal densitometry annulus and layer averages).

The Pentcam system (Oculus GmBH, Germany) provides a topical graphical map (Figure 6, top right; the Cornea Densito Average) and a set of numbers in gray scale units for different locations (Figure 6, middle right, Corneal densitometry annulus and layer averages). Overall, therefore, the Pentacam instrument can be set up so as to assess the amount of backscattered light in the form of arbitrary density units (called gray scale units, GSU), i.e. with no detectable backscatter, the reported density would be zero with the top end to the scale being 100. This principle underlies the assessment of corneal densitometry with the Pentacam imaging system, that can assess corneal thickness at the same locations as

backscattered light is assessed. For this newer application of Scheimpflug imaging, a special type of rotating illumination is projected onto the cornea and is considered useful to reduce variability in the corneal profile that can be seen. This is because very large numbers of measurements are made to define the location of the anterior and posterior surfaces [28,29], i.e. overcoming the alignment difficulties noted in earlier studies on corneal light scattering [16].

It is very important to note that the value reported for the so-called 'anterior densitometry' is not a simple measure of epithelial light scatter but rather is of the backscatter data derived from the anterior 120 µm (0.120 mm) of the corneas. This anterior zone comprises just over 1/5th of the expected average thickness value for the central region of a healthy human cornea (i.e. the 0.535 mm value generated from analyses of data from 300 different reports on white (Caucasian) eyes [39]) but is set at the same value regardless of the central corneal thickness (CCT) value. There are three inter-related aspects of this anterior zone selection that need to be considered.

Firstly, the anterior zone selected in the Pentacam system extends well beyond the actual corneal epithelium, for which measurements by OCT indicate a thickness of c. 45 to 50 µm (i.e. 0.045 to 0.050 mm) [30,68]. Secondly, this 120 µm (0.120 mm) zone selected for the central corneal region is presumably reasonably constant (in terms of the instrument's ability to define different optical layers within the cornea) but regardless of this it will represent a variable proportion of the total thickness value according to an individuals actual CCT value. So, for example, in the meta-analysis of published data on white (Caucasian, no-Asian) individuals it was estimated that normal CCT could be anywhere between 0.475 and 0.597 mm (based on 1.96 SD for the averaged CCT value of 0.535 mm) [39]. The anterior 0.120 mm zone could thus represent between 25 and 20 % of the total CCT as a first approximation. That

this is the case is because CCT is more usually taken to be a point value (including with the Pentcam, where this is over a region of just 0.5 mm diameter) and may be the same 'size' as the densitometry value indicated on the 'Cornea Densito Average' topographic map. In contrast, the optical densitometry central zone for which the annulus data is generated is 2 mm in diameter. Thirdly, the issue of the relative contribution made by the anterior aspect of the cornea is even more complex once non-central regions are considered. Modern-day optical imaging of the cornea with the Orbscan or Pentacam systems have provided compelling evidence that there are predictable increase in corneal thickness when comparing mid-peripheral and peripheral locations to the central region [31,69]. While different investigators have not always used the same approaches to calculate the progressive thickening of the peripheral cornea, the results are generally consistent and it so its is important to recognize that essentially nothing is known about the transition from 'anterior' to middle to posterior aspects of the cornea from a structural and ultrastructural perspective. So, for example, the 'anterior stroma' considered in electron microscopy-based modelling studies on light scattering was based on measures made of fibrils immediately underneath the epithelium [8] and so is very different to the variable anterior 0.120 mm layer assessed by the densitometry measures in the Pentcam.

Overall, for normal adults, the measured densitometry (based on very low amounts of backscattered light) is expected to be low [28], but in the presence of acute bacterial keratitis [28] or in substantial corneal clouding diseases such as mucopolysaccharidosis (MPS) [70] Pentacam-based studies have revealed substantial increases in corneal densitometry values where distinctive and substantial haze in the cornea is evident clinically. While it might well be argued that one does not need a Pentacam-type system to facilitate diagnosis of such diseases, sensitive quantitative measures of the optical density of a corneal graft (in an MPS

patient) could be very useful to establish – at the earliest opportunity – whether or not the dystrophy was recurrent. Such sensitivity to objective optical measures should also be relevant to assessing the long-term effects of contact lens wear on the cornea.

Corneal thickness differences or changes are a possible characteristic of adjustment to contact lens wear [4,34-37,45,46,63,75,76], but any one consequence of long term contact lens wear could be the development of discrete opacities [54-62]. Such effects may be very regional, e.g. affecting more the posterior stroma rather than the anterior stroma. Therefore, a general question can be asked as to whether these might be detectable with the Pentacam and how these might relate to any differences in corneal thickness. The literature on this is rather scant with inconclusive results reported [71-74]. The possible association between these two variables could be important with, for example, an increased densitometry being a predictable consequence of the development of discrete opacities in the corneal stroma (with posterior striae perhaps also evident). However, a case could also be made that if there was (substantial oedema) then the overall number of opacities per unit area could be observed to be lower as they would be more spread out.

7. Summary and conclusions

This review will hopefully provide a useful summary of the changes in perspectives on corneal transparency, especially in relation to its subjective and objective assessment in contact lens wearers. There has been interest in the assessment of corneal transparency and / or the development of oedema for over 50 years and a recurrent issue has been the localization of the swelling and its structural (anatomical) cause. This issue is still unresolved with there having been quite considerable differences in literature reports linked to this. A user-friendly option (for the clinician in routine practice) is now available to assess changes in optical

transparency with contact lens wear and even on its location. The instrument output is very substantial with details available for the reduced transparency (as increased densitometry) across a small central region as well as from the mid-peripheral and even peripheral cornea. Furthermore, such densitometry assessments can also be optically- segmented into layers so that either the anterior or posterior transparency can be objectively assessed. At the present time, there are no obvious guidelines available on whether only selected data should be provide in such assessments, as opposed to simply reporting all of the output in full detail. It remains to be established how any regional differences in optical densitometry can be related to other instrument- or patient-related assessments of changes in optical transparency of the cornea. Clarification of any such possible inter-relationships will also require systematic assessments to be made of each aspect of the reported densitometry data to establish, for example, which are the most predictable and reproducible.

Acknowledgements

The protocols for all clinical images included in this review were approved by the university-based ethics committee. No specific funding was procured for these evaluations or the writing of this review.

Competing interests

The authors have no proprietary interests in any of the equipment or procedures considered in this review and report no conflicts of interest.

References

1. Doughty MJ. Comparative anatomy and physiology of the cornea and conjunctiva. In Ocular Surface (Martin Herran R, Corrales RM, eds). CRC Press – 2012; pp. 32-78.
2. Doughty MJ. A physiological perspective on the swelling properties of the mammalian corneal stroma. Contact Lens Anterior Eye 2003; 26: 117-129.
3. Montés-Micó R. Role of the tear film in the optical quality of the human eye. J Cataract Refract Surg. 2007; 33: 1631-1635.
4. Schoessler JP, Lowther GE. Slit lamp observations of cornea edema. Am J Optom Arch Am Acad Optom. 1971; 48: 666-671.
5. O'Donnell C, Wolfssohn JS. Grading of corneal transparency. Contact Lens Anterior Eye 2004; 27: 161-170.
6. Maurice DM. The structure and transparency of the cornea. J Physiol. 1957; 136: 263-286.
7. Bergmanson JPG, Horne J, Doughty MJ, Garcia M, Gondo M. Assessment of the number of lamellae in the central region of the normal human corneal stroma, at the resolution of the transmission electron microscope. Eye Contact Lens 2005; 31: 281-287.
8. Freund DE, McCally RL, Farrell RA, Cristol SM, L'Hernault NL, Edelhausrer HF. Ultrastructure in anterior and posterior stroma of perfused human and rabbit corneas. Invest Ophthalmol Vis Sci. 1995; 36: 1508-1523.
9. Maurice DM, Giardini AA. Swelling of the cornea in vivo after the destruction of its limiting layers. Br. J. Ophthalmol. 1951; 35: 791-797.
10. Potts AM, Friedman BC. Studies on corneal transparency. Am J Ophthalmol. 1959; 48(Pt.2): 480-487.

11. Mandell RB, Polse KA. Corneal thickness changes as a contact lens fitting index – experimental results and a proposed model. Am J Optom Arch Am Acad Optom. 1969; 46: 479-491.

12. Jester JV, Moller-Pedersen T, Huang J, Sax CM, Kays WT, Cavanagh HW, Petroll WM, Piatigorsky J. The cellular basis of corneal transparency: evidence for 'corneal crystalline'. J Cell Sci.1999; 112: 613-622.

13. Walsh JE, Bergmanson JPG, Koehler LV, Doughty MJ, Fleming D, Harmey JH. Fibre optic spectrophotometry for the *in vitro* evaluation of ultraviolet radiation (UVR) spectral transmittance of rabbit corneas. Physiological Meas. 2008; 29: 375-388.

14. Tsukahara N, Tani Y, Kikuchi H, Sugita S. Light transmission of the ocular media in birds and mammals. J Vet Med Sci. 2014; 76: 93-95.

15. Zinflou C, Rochette RJ. Ultraviolet A-induced oxidation in corneas: Characterization of the early oxidation-related events. Free Rad Biol Med. 2017; 108: 118-128.

16. Olsen T. Light scattering from the human cornea. Invest Ophthalmol Vis Sci. 1982; 23: 81-86.

17. Li HF, Petroll WM, Moller-Pedersen T, Maurer JK, Cavanagh HD, Jester JV. Epithelial and corneal thickness measurement by in vivo confocal microscopy through focussing (CMTF). Curr Eye Res. 1997; 16: 214-221.

18. Efron N, Perez-Gomez I, Mutalib HA, Hollingsworth J. Confocal microscopy of the normal human cornea. Contact Lens Anterior Eye 2001; 24: 16-23.

19. Jalbert I, Stapleton F, Papas E, Sweeney DF, Coroneo M. In vivo confocal microscopy of the human cornea. Br J Ophthalmol. 2003; 87: 225-236.

20. Patel SV, Mclaren JW, Hodge DO, Bourne WM. Confocal microscopy in vivo in corneas of long term contact lens wearers. Invest Ophthalmol Vis Sci. 2002; 43: 995-1003.

21. McLaren JW, Wacker K, Kane KM, Patel SV. Measuring corneal haze by using Scheimflug photography and confocal microscopy. Invest Ophthalmol Vis Sci. 2016; 57: 227-235.

22. Moller-Pedersen T. Keratocyte reflectivity and corneal haze. Exp Eye Res. 2004; 78: 553-560.

23. Doughty MJ, Seabert W, Bergmanson JPG, Blocker Y. A descriptive and quantitative study of the keratocytes of the corneal stroma of the albino rabbit using transmission electron microscopy. Tissue Cell 2001; 33: 408-422.

24. Doughty MJ, Bergmanson JPG. Use of a stroma perfusion technique and transmission electron microscopy to assess ultrastructural changes associated with exposure to slightly acid pH 5.75 solutions. Curr Eye Res. 2008; 33: 45-57.

25. Maldonado MJ, Arnau V, Martinez-Costa R, Navea A, Mico FM, Cisneros AL, Menezo JL. Reproducibility of digital iamage analysis for measuring corneal haze after myopic photorefractive keratectomy. Am J Ophthalmol. 1997; 123: 31-41.

26. Polunin GS, Kourenkov VV, Polunina EG. Corneal transparency and measurement of corneal permeability in excimer laser photorefractive ketatectomy. J Refract Surg. 1988;14: S230-234.

27. Chan JW, Edwards MH, Wood GC, Woo VC. Objective method to measure corneal clarity before and after laser in situ keratomileusis. J Cataract Refract Surg. 2003; 29: 118-124.

28. Nagel S, Wiegand W, Thaer AA, Geyer O-C. Streulichtuntersuchung der Kornea bei Kontaklinsenträgern. In-vivo-Untersuchungen mittels der konfokalen Spalt-Scanning-Mikroskopie. Ophthalmologe 1996; 93: 252-256.

29. Ni Dhubhghaill SN, Rozema JJ, Jongenelen S, Hidalgo IR, Zakaria N, Tassignon M-J. Normative values for corneal densitometry analysis by Scheimpflug optical assessment. Invest Ophthalmol Vis Sci. 2014; 55: 162-168.

30. Otri A, Fares U, Al-Aqaba MA, Dua H. Corneal densitometry as an indicator of corneal health. Ophthalmology 2012; 119: 501-508.

31. Lee M, Ahn J. Effects of central stromal thickness and epithelial thickness on intraocular pressure using Goldmann applanation and non-contact tonometers. PLos One 2016; 11: e0151868.

32. Jonuscheit S. Data extraction and reporting strategies of studies assessing non-central corneal thickness by Pentcam: a review. Contact Lens Anterior Eye 2014; 37: 323-330.

33. Wilson MS. Corneal edema from contact lens wear, its causes and treatments. Trans Ophthamol Soc UK.1970; 90: 31-45.

34. Cox I, Holden BA. Can vision loss be used as a quantitative assessment of corneal edema. Int Contact Lens Clin. 1990; 17: 176-179.

35. Lambert SR, Klyce SD. The origin of Sattler's veil. Am J Ophthalmol. 1981; 91: 51-56.

36. Griffiths SN, Drasdo N, Barnes DA, Sabell AG. Effect of epithelial and stromal edema on the light scattering properties of the cornea. Am J Optom Physiol Opt. 1986; 63: 888-894.

37. Elliott DB, Fonn D, Flannagan JG, Doughty MJ. The relative sensitivity for clinical tests to hydrophilic lens-induced corneal thickness changes. Optom Vis Sci. 1993; 70: 1404-1408.

38. Mishima S. Corneal thickness. Surv Ophthalmol. 1968; 13: 57-96.

39. Doughty MJ, Zaman ML. Human corneal thickness and its impact on intraocular pressure measures: A review and meta-analysis approach. Surv Ophthalmol. 2000; 44, 367-408.

40. Khoramnia R, Rabsilber TM, Auffarth GU. Central and peripheral pachymetry measurements according to age using the Pentacam rotating Scheimpflug camera. J Cataract Refract Surg.2007; 33:830–836

41. Zheng Y, Huang G, Huang W, He M. Distribution of central and peripheral corneal thickness in Chinese children and adults: the Guangzhou twin eye study. Cornea 2008; 27: 776-781

42. Galgauskas S, Juodkaite G, Tutkuviene J (2014) Age-related changes in central corneal thickness in normal eyes among the adult Lithuanian population. Clinical Interventions Aging 2014; 9: 1145-1151.

43. Hashemi H, Asgari S, Mehravaran S, Emamian SH, Shariati M, Fotouhi A. The distribution of corneal thickness in a 40- to 64-year old population of Sharroud, Iran. Cornea 2011; 30: 1409-13.

44. Mandell RB, Polse A, Fatt I. Corneal swelling caused by contact lens wear. Arch Ophthalmol. 1970; 83: 3-9.

45. Liu Z, Pflugfleder SC. The effect of long-term contact lens wear on corneal thickness, curvature and surface regularity. Ophthalmology 2000; 107: 105-111.

46. Myrowitz EH. Melia M, O'Brien TP. The relationship between long-term contact lens wear and corneal thickness. CLAO J. 2002; 28: 217-220.

47. Carney LG, Jacobs RJ. Mechanisms of visual loss in corneal oedema. Arch Ophthalmol. 1984; 102: 1068-1071.

48. Yong C, Xuan S, Xiao-guang C, Xiao-xin LI, Yong-zhen B. Correlation between contrast sensitivity and the lens opacitiesclassification system III in age-related nuclear and cortical cataracts. Chin Med J. 2013; 126: 1430-1435

49. Owsley C, Sekuler R, Siemsen D. Contrast sensitivity throughout adulthood. Vision Res. 1983; 23: 689-699.

50. Applegate RA, Massof RW (1975) Changes in contrast sensitivity function induced by contact lens wear. Am J Optom Physiol Opt. 1975; 52: 840-846.

51. Nowozyckyj A, Carney LG, Efron N. Effect of hydrogel lens wear on contrast sensitivity. Am J Optom Physiol Opt. 1988; 65: 263-271.

52. Thai LC, Tomlinson A, Ridder WH. Contact lens drying and visual performance: the vision cycle with contact lenses. Optom Vis Sci. 2002; 79: 381-388.

53. Lira M, Dos santos Castenheira EM, Santos L, Azeredo J, Yebra-Pimentel E, Real Oliveira MECD. Changes in UV-visible transmittance of silicone-hydrogel contact lenses induced by wear. Optom Vis Sci. 2009; 86: 332-339.

54. Holland EJ, Lee RM, Bucci FA, Janda AM, Doughman DJ, Harris JK, Krachmer JH. Mottled cyan opacification of the posterior cornea in contact lens wearers. Am J Ophthalmol. 1995; 119: 620-626.

55. Remeijer L, Van Rij G, Beekhuis WH, Polak BC, Van Nes J. Deep corneal stroma opacities in long-term contact lens wear. Ophthalmology 1990; 97: 281-285.

56. Göbbels M, Wähning A, Spitznas M. Endothelfunktion bei kontaklinsenbedingten tiefen Hornhauttrubüngen. Forstr. Ophthalmol. 1989; 86: 448-450.

57. Brooks AM, Grant G, Westmore R, Robertson IF. Deep corneal stromal opacities with contact lenses. Aust NZ J Ophthalmol. 1986; 14: 243-249.

58. Rubey F, Harrer S. Hornhautveränderungen verursacht durch HEMA-Linsen. Klin Monatsbl Augenheilkd. 1982; 181: 344-345.

59. Bohnke M, Masters BR. Long term contact lens wear induces a corneal degeneration with microdot deposits in the corneal stroma. Ophthalmology 1997; 104: 1887-1896.

60. Hollingsworth JG, Efron N. Confocal microscopy of the corneas of long-term rigid contact lens wearers. Contact Lens Anterior Eye 2004; 27: 57-64.

61. Trittibach P, Cadez R, Eschmann R, Saara G-M, Boehnke M, Frueh BE. Determinants of microdot stromal degenerations within corneas of long-term contact lens wearers by confocal microscopy. Eye Contact Lens 2004; 30: 127-131.

62. Bastion M-LC, Mohamad MH. Study of the factors associated with the presence of white dots in the corneas of regular soft contact lens wearers from an Asian country. Eye Contact Lens 2006; 32: 223-227.

63. Doughty MJ. An observational cross-sectional study on the corneal endothelium of medium-term rigid gas permeable contact lens wearers. Contact Lens Anterior Eye 2017; 40: 109-115.

64. Patel S, Winter EJ, McLaren JW, Bourne WM. Objective measurement of backscattered light from the anterior and posterior cornea in vivo. Invest Ophthalmol Vis Sci. 2007; 48: 166-172.

65. Wang J, Simpson TL, Fonn D. Objective measurements of corneal light-backscatter during corneal swelling by optical coherence tomography. Invest Ophthalmol Vis Sci. 2004; 45: 3493-3498.

66. Caldicott A, Charman WN. Diffraction haloes from corneal oedema and epithelial cell size. Ophthal Physiol Opt. 2002; 22: 209-213.

67. Smith GT, Brown NA, Shun-Shin GA. Light scatter from the central human cornea. Eye 1990; 4: 584-588.

68. Kim BJ, Ryu I-H, Kim SW. Age-related differences in epithelial thickness measurements with anterior segment optical coherence tomography. Jpn J Ophthalmol. 2016; 60: 357-364.

69. Jonuuscehit S, Doughty MJ, Button NF. On the use of Orbscan II to assess peripheral corneal thickness in humans – a comparison with ultrasound pachometry measures. Ophthal Physiol Opt. 2007; 27: 179-189.

70. Elflein H, Hofherr T, Berisha-Ramadani F, Weyer V, Lampe C, Beck M, Pitz S. Measuring corneal clouding in patients suffering from mucopolysaccharidosis with the Pentacam densitometry programme. Br J Ophthalmol. 2013; 97: 829-833.

71. Anayol MA, Sekeroglu MA, Ceran BB, Dogan M, Gunaydin S, Yilmazbas P. Quantitative assessment of corneal clarity in keratoconus: a case control study of corneal densitometry. Eur J Ophthalmol. 2016; 26: 18-23.

72. Pekel G, Firinci F, Acer S, Kasikçi S, Yagci R, Mete R, Cetin EN. Optical densitometric measurements of the cornea and lens in children with allergic rhinoconjunctivitis. Clin Exp Optom. 2016; 99: 51-55.

73. Pekel G, Özbakis F, Bahar A, Pekel E, Çetin EN. Correlations of corneal optical densitometry, endothelial hexagonality percentage, and epithelium thickness. Curr Eye Res. 2018; 43: 170-174.

74. Garzón N, Poyales F, Illarramendi I, Mendicute J, Jáñez O, Caro P, López A, Argueso F. Corneal densitometry and its correlation with age, pachymetry, corneal curvature, and refraction. Int Ophthalmol. 2017; 37: 1263-1268.

75. Rosenthal P. Corneal contact lenses and edema. Int Ophthalmol Clin. 1968; 8(3): 611-621.

76. Erickson P, Comstock TL, Doughty MJ, Cullen AP. The corneal swells in the posterior direction under hydrogel contact lenses. Ophthal Physiol Opt. 1999; 19: 475-480.